LDH nanoparticles: synthesis, size control and applications in nanomedicine

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Abstract

Due to their negligible toxicity and buffering properties, layered double hydroxides (LDHs) are commercially employed as antacids and they are increasingly studied as vehicles in drug and gene delivery systems. The bioactive compounds (drugs, biomolecules) are incorporated by intercalation or adsorption and released under appropriate conditions by anion exchange, desorption and LDH layers dissolution. More recently, their application as nanovehicles for cellular delivery has been developed based on their synthesis in the nanometer scale. LDH nanoparticles are obtained by different synthesis routes, but the more extended involves coprecipitation at either constant or variable pH and Hydrothermal aging in pure water. LDH based nanocarriers present several advantages as drug and gene nanovehicles when compared to other inorganic nanoparticles. First, LDH nanovehicles interact with the negatively charged cell membrane due to the positive charge and the hydrophilic character of the nanoparticles and their transfection is mainly produced by clathrin-mediated endocytosis. Secondly, LDH nanovehicles are dissolved in mildly acid media into non-toxic species, releasing their cargo and preventing the accumulation usually observed with polymers and other inorganic vehicles. Finally, they are expected to avoid renal clearance and to allow long circulation times due to their size. In this chapter, the strategies to obtain LDH nanoparticles as well as the factors that determine the particle size distribution are reviewed with emphasis on their application in drug and gene delivery. Hence, the chapter highlights the physicochemical factors that affect the delivery process and the proposed customization strategies to produce an optimal performance of LDH nanoparticles as drug nanovehicles.

1. Introduction

The continuous development of new drugs, especially biomolecules such as proteins or DNA, has leaded to great advances in public health. Nevertheless, these therapeutic agents are distributed according to their physicochemical properties (charge, solubility, hydrophobicity), which results in an inefficient distribution and undesirable or even harmful side-effects. Moreover, drugs and especially biomolecules can be degraded in the biological media before reaching their therapeutic target, diminishing their effectiveness. For all these reasons, there is an increasing demand of new drug nanocarriers that protect the pharmaceutical agents and release them selectively in the therapeutic target. In the last years, the spectacular advance in the nanoscience field together with the unique properties of nanoparticles (high surface/volume ratio, optical and magnetic properties, among others) have generated new systems of multifunctional therapeutics. However, aggregation control and custom surface properties are mandatory to avoid nanocarriers

elimination by the immune system or to increase their cellular transfection efficiency. Moreover, in order to design nanocarriers that bestow site specific release it is necessary, not only a precise selection of the drug and the nanocarrier, but also a (bio)functionalization strategy to optimize their selectivity towards the therapeutic target [1,2].

Among the systems studied as nanocarriers, much attention has been given to liposomes, polymeric and metallic nanoparticles, intercalation compounds, oxides [1–3] and layered double hydroxides (LDHs) [4–6]. These last solids are non-toxic easily, and inexpensively prepared, and present easily customizable physicochemical properties, anion exchange capacity and weathering reactions. The structure of LDHs is derived from that of brucite ($Mg(OH)_2$), which can be described as a close packing of hydroxyl anions where half octahedral sites (all of one layer, none of the next) are occupied by Mg^{2+} ions. In the case of LDHs, a positive charge excess is produced by isomorphic substitution of divalent by trivalent ions, and compensated by the introduction of anions between the layers, which share the interlayer space with solvent molecules (usually water). The general formula of these solids can be written as: $[M^{II}_{1-x}M^{III}_{x}(OH)_2]A^{n-}_{x/n}\cdot mH_2O$, where M^{II} = Mg^{2+}, Zn^{2+}, Ni^{2+} ..., M^{III} = Al^{3+}, Fe^{3+}, Cr^{3+}..., A^{n-} = CO_3^{2-}, SO_4^{2-}, Cl^-. In the case of biomedical aplications, Mg^{2+} and Al^{3+} are usually constituting the layers and the drug can be incorporated as the interlayer anion (A^{n-}) or on the particle surface.

LDHs present applications in fields such as catalysis, environmental remediation, plastics and pharmaceutics [7–12]. Their biocompatibility and acid-base properties have leaded to market formulations as antacids (i. e., Talcid ® and Almax®) and they are also increasingly studied as drug carriers to deliver compounds such as non-steroidal antiinflamatories (NSAIDs), antibiotics, bone resorption regulators, antihypertensives or anticancerigens [4,6,13–15]. Further, biomolecules like vitamins, DNA, siRNA and proteins have also been incorporated to these solids, leading to potential applications in cosmetology, cancer therapy and vaccination, among others [16–18].

2. Synthesis and size-modulation of LDH nanoparticles.

2.1. Traditional synthesis methods: coprecipitation.

LDHs are easily and inexpensively prepared at laboratory scale, but appropriate synthesis routes and conditions are needed to obtain multifunctional LDH systems with a fine tuning of their properties [19]. Coprecipitation is one of the most usual methods because of its simplicity, flexibility and capacity to produce solids with different composition and physicochemical properties [20,21]. This method involves mixing solutions of the metal ions and the hydroxyl anions in the presence of a salt of the interlayer anion. It is performed at constant pH [20,22] (or low supersaturation conditions) when the metal ions and the base solutions are added slow and simultaneously to a solution containing the interlayer anion. The addition rate of both solutions is controlled to keep a constant pH. On the contrary, it is performed at variable pH [23] (or high supersaturation conditions) when either the base or the metal ions are dissolved with the interlayer anion, and the remaining reagent is added without any pH control. In all cases, the synthesis is performed at basic pH values and, consequently, N_2 purging is necessary to avoid intercalation of carbonate, which present high affinity for LDH layers. Coprecipitation at constant

pH produce larger and more crystalline particles than at variable pH. The slurries obtained by either of these methods are aged under hydrothermal conditions to increase the crystallinity and particle size of the solids. Other synthesis methods include homogeneous precipitation by urea hydrolysis [24,25], salt-oxide and sol-gel reactions, etc. [19]. Although the particles obtained by all these methods are too large for nanomedicine applications, new synthesis methods have been introduced in the last years to produce LDH nanoparticles suitable for such applications.

2.2. Separate nucleation and aging steps: an easy way to go from micro to nano.

Zhao and coworkers [26] reported the first synthesis of LDH nanoparticles, which involved fast mixing of the metal salts and alkaline solutions in a colloid mill. After a short equilibration time, the obtained solid was separated from the supernatant and washed several times with water. Then, the remaining solid was dispersed in pure water and aged under hydrothermal conditions. The produced carbonate intercalated LDHs showed similar composition and structure to that one synthesized by a typical coprecipitation method at constant pH. However, the solid obtained by this method presented lower particle size, higher crystallinity and higher lateral to thickness ratio than those obtained by classical coprecipitation methods. This work set the standard for many synthesis methods pointing at obtaining LDH nanoparticles: a nucleation step that involves a fast mixing of the alkaline and the metal salts solutions followed by an aging step performed in a separate dispersion of LDH nanoparticles in pure water under hydrothermal conditions. The fast mixing of the reactants is intended to produce a faster nucleation than crystal growth, leading to a size diminution of the platelets. On the other hand, aging of LDH nanoparticles in pure water produces a minimal aggregation due to the low ionic strength and high thermal energy.

The most common variation of this method was introduced by Xu et al. [27], who simplified the nucleation step by using a fast coprecipitation method at variable pH. Briefly, a solution containing the metal salts was poured into the alkaline solution under vigorous stirring and the obtained solid was immediately centrifuged, washed and aged in pure water. An The Mg/Al ratio in the initial metal ions solution was 3:1, but the amount of NaOH contained in the alkaline solution was just enough to precipitate a 2:1 Mg-Al-LDH. The Mg^{2+} excess hindered the formation of undesirable phases of non-lamellar Mg-Al double hydroxides and gibbsite. The freshly prepared dispersions showed bimodal particle size distributions with maxima around 300 and 2000 nm. After hydrothermal treatment in pure water for 4 hours at 100 ºC the agglomeration was diminished, leading to a single size distribution centered around 100 nm.

As an alternative to this method, we recently obtained LDH nanoparticles by precipitation at constant pH. Briefly, a 100 mL solution (0.3 M $MgCl_2$, 0.1 M $AlCl_3$) was added dropwise to a 200 mL, 0.1 NaCl solution while the pH was set constant to 9 by controlled addition of 2 M NaOH solution. After equilibration for 1 hour, the solid was separated from the mother liquor, washed twice and redispersed in water. The dispersion was agitated for 24 hours and then aged under hydrothermal conditions (80 ºC, 24 hours). In order to obtain pure phases of Mg-Al LDHs, the Mg/Al ratio was 3:1 and the pH was set to a pH value where the precipitation of $Mg(OH)_2$ was complete. Otherwise, the partial dissolution of $Mg(OH)_2$ during the washing and aging steps caused the formation of undesired phases of $MgAl_2(OH)_8$, a double hydroxide with a non-layered

structure (JCPDS card number 35-1274). The size distribution of LDH particles was determined in the different steps of the process (Figure 1) by dynamic light scattering in a Delsa Nano C instrument (Beckman Coulter). The size distribution of the particles right after the synthesis (as prepared), before the separation of the supernatant, was wide due to the high ionic strength produced by the synthesis reaction, which can be written as:

$$3MgCl_{2(aq)} + AlCl_{3(aq)} + 8NaOH_{(aq)} + nH_2O_{(l)} \rightarrow Mg_3Al(OH)_8Cl \cdot nH_2O_{(s)} + 8NaCl_{(aq)}$$

After the solid washing and dispersion in pure water at room temperature (25 ºC) for 24 hours, the width and the average diameter of the nanoparticles was diminished, indicating that the ionic strength diminution lead to a lower agglomeration. The disaggregation of the particles was even better by aging in hydrothermal conditions (80 ºC, 24 hours), and a narrow particle size distribution centered around 100 nm was obtained. This dispersion was stable against agglomeration, the particle size being unchanged for at least a month. The low aggregation and high stability of the LDH nanoparticles dispersions prepared by these methods are related to their high positive zeta potential (above 40 mV in most cases), leading to strong particle-particle repulsions.

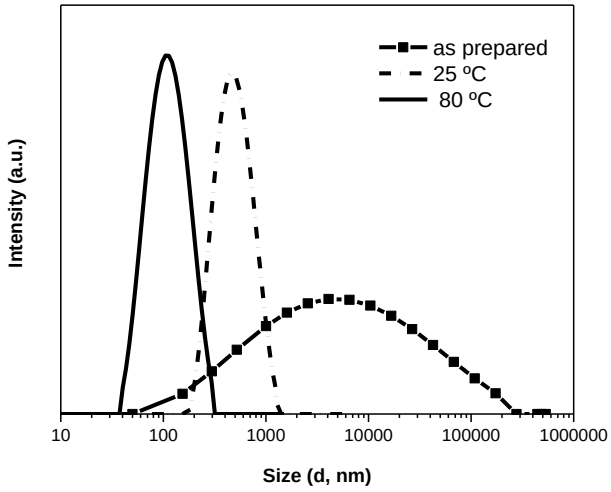

Figure 1. Size distribution of chloride intercalated LDHs prepared by coprecipitation at pH 9 with a 3:1 Mg/Al ratio: as prepared, after aging at room temperature (25 ºC) and hydrothermal treatment (80 ºC) for 24 hours

The SEM images of these solids (Figure 2A) showed regular particles with hexagonal symmetry and defined edges for both chloride and carbonate intercalated LDHs. Compared to particles obtained by the conventional coprecipitation process followed by aging in the mother liquor, the particle size was strongly diminished and the symmetry of the particles increased.

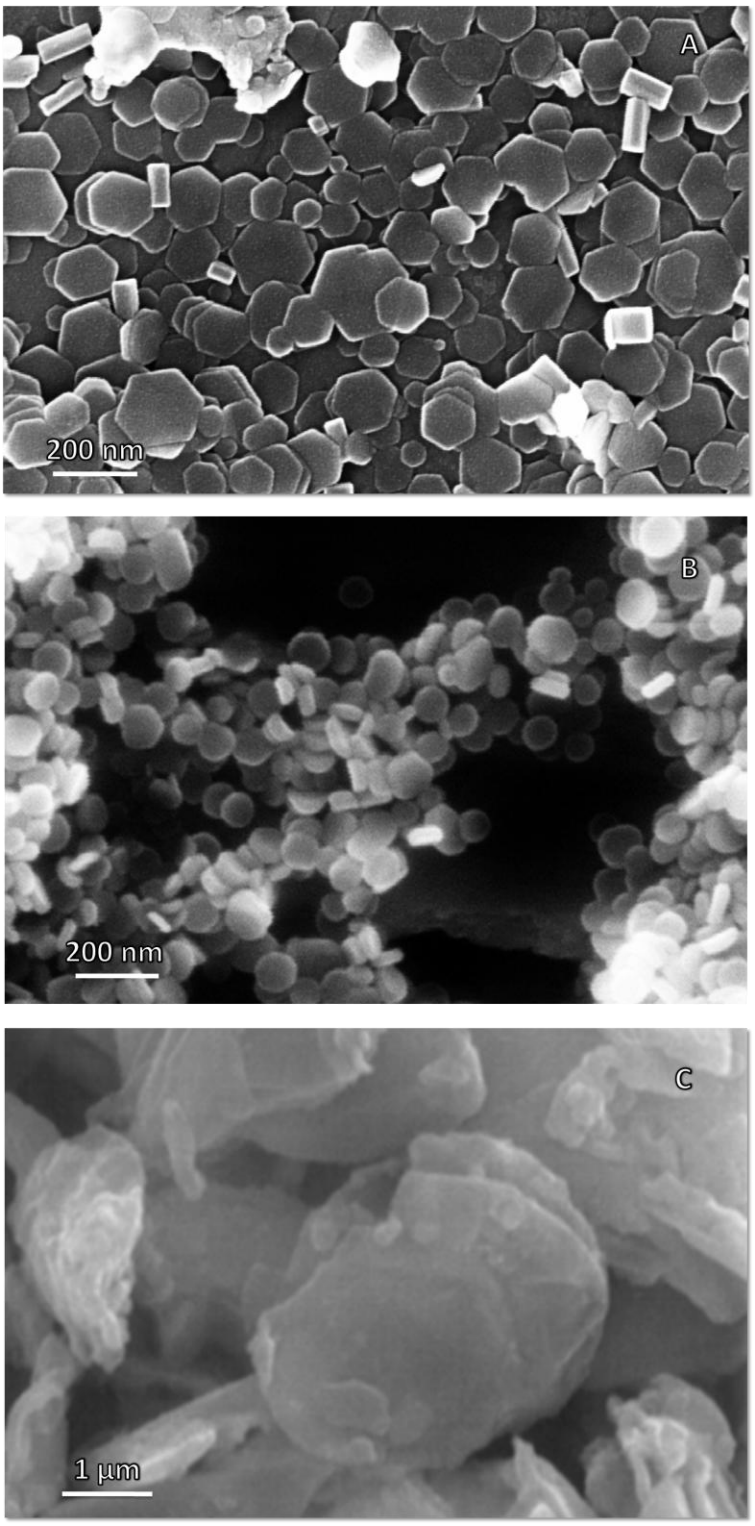

Figure 2. SEM images of chloride (A) and carbonate (B) intercalated LDHs prepared by the coprecipitation method at constant pH followed by separate aging. (C) carbonate intercalated LDH aged in the supernatants resulting from the nucleation step.

The drug loading is incorporated to LDH nanoparticles either in the interlayer space and/or on the particle surface during the synthesis or by anion exchange in a separate step. Chloride or nitrate

intercalated nanocarriers are chosen for the latter process, as they present weak electrostatic interactions with the positively charged layers. Anionic drugs (NSAIDs, methotrexate -MTX-, among others), especially those with a simple molecular structure, are usually incorporated to both the interlayer and the surface of LDH nanocarriers [28–30]. On the other hand, anionic biopolymers, such as proteins, siRNA or DNA are mainly incorporated to the particle surface and partially exchanged at the edges of the particles. Consequently, the loading capacity of the LDH nanocarrier is increased as the particle size decreased due to the increased surface area of both basal planes and edges [31,32].

Furthermore, combined delivery routes have been described with an anticancer drug (5 fluoruoracil, 5-FU) and a gene silencing agent (Allstars Cell Death siRNA), loaded in the interlayer and on the surface of LDH nanoparticles, respectively [29]. First, 5-FU was incorporated by anion exchange with a chloride containing LDH nanocarrier obtained by the fast coprecipitation method and separate aging. Afterwards, the siRNA was adsorbed on the surface of the 5-FU/LDH nanohybrids. The prepared nanohybrids caused larger cell death than similar concentration of nanohybrids containing only one of the anticancer agents, indicating a synergistic effect between them.

Finally, the intercalation method also affects the morphology of the particles. For example, two types of LDH nanoparticles labeled with flourescein isothianate (FITC) have been obtained by anion exchange [33]. Rod (30-60 nm wide, 100-200 nm long) and hexagonal (50-150 nm lateral size) shaped nanoparticles were obtained when the anion exchange step was performed before or after the aging step.These particles exhibit different cellular compartment depending on their morphology (see below).

2.3. Controlling the size of LDH nanoparticles.

Controlling the particle size of LDH nanoparticles is of paramount importance as this parameter determines their citotoxicity [34], cellular transfection efficiency [31,35], and drug release profile [28]. The size of LDHs can be tailored by controlling synthesis conditions either in the nucleation or the aging step (Table 1). The solid composition, especially the interlayer anion, also determines the size of the nanoparticles (Table 2).

Changes in the experimental conditions that lead to a lower LDH solubility during the nucleation step, such as temperature or solvent, produce a size diminution of the LDH nanoparticles. Thus, Dong et al [31,35,36] proposed a synthesis method in methanol that involved nucleation by drop-wise addition of the metal salt solution to the base solution and separate aging under hydrothermal conditions in pure methanol. The final solid was collected, washed and dispersed in water. The particle size diminished slowly during the first 4-6 days, and reached a particle diameter below 50 nm and a narrow size distribution. The particle size was smaller than that obtained in water, which was assigned to the lower solubility of LDHs in methanol that caused a faster crystal nucleation and the formation of numerous, small nuclei. Similarly, Ladewig et al [37] studied the influence of temperature during the nucleation step in the separate nucleation and

aging method. A temperature diminution leaded to the formation of smaller LDH particles, which was assigned to a solubility decrease with diminishing temperature.

Temperature and time are important factors to determine the size of LDH nanoparticles during the aging step (Table 1). Thus, Oh et al. [38] obtained 50 nm carbonate-intercalated LDH nanoparticles in the absence of hydrothermal treatment, while 100, 200, and 300 nm particles were prepared by hydrothermal treatment in different conditions. Other authors reported similar sequences for LDHs containing other interlayer anions and at different Mg/Al ratios (Table 1). Xu et al. [27] proposed that, besides disaggregation of LDH nanoparticles, also a 3D process (disaggregation of small crystallites, diffusion of the products and deposition on larger crystallites) was concurrent in the aging step. This process leaded to particle growth that also enhanced reaggregation due to the stronger interactions between the resulting particles.

Table 1: Average particle diameter of LDH nanoparticles prepared at different aging conditions.

Composition	**Mg/Al Ratio**	**Hydrotermal treatment**	**Diameter (nm)**	**References**
MgAl-Cl-LDH	2:1	100° 16 hours	85	[33]
	7:3	100° 16 hours	106	[39]
	3:1	100° 8 hours	101	[27]
		100° 16 hours	114	[27]
		100° 48 hours	160	[27]
		100° 144 hours	284	[27]
MgAl-NO_3-LDH	2:1	150° 24 hours	100	[40]
	3:1	100° 8 hours	120	[37]
		100° 16 hours	120	[37]
		100° 24 hours	125	[37]
		100° 48 hours	145	[37]
MgAl-CO_3-LDH	2:1	none	50	[38]
		100° 12 hours	100	[38]
		200° 24 hours	200	[38]
		200° 48 hours	350	[38]
	3:1	100° 8 hours	58	[27]
		100° 16 hours	71	[27]
		100° 72 hours	118	[27]

Finally, the particle size is marginally changed when varying the layer-constituting ions but the interlayer anion is an important factor (Table 2) that affects the layer stacking and size of the LDH particles, as can be observed in the lateral size and thickness of carbonate and chloride intercalated LDHs included in figures 2A and 2B. However, the main effect of the interlayer anion is related to the surface charge of LDH nanocarriers [20,41–44]. It is well known that controlling the zeta potential is an effective tool to achieve an optimal dispersion and a minimum agglomeration of nanoparticles in general [45]. Chloride-intercalated LDH nanoparticles exhibit a zeta potential of 30-50 mV, which is the main reason to their easy dispersion by increasing the thermal energy. Nevertheless, the zeta potential of LDH nanoparticles can be reverted by the

incorporation of anions such as dodecylsulfate [20] or ibuprofen [8,30] that , besides electrostatic interactions, present chemical affinity for the LDH surface. Further, the zeta potential of LDH particles is highly sensitive to the interaction with species such as surfactants, polymers, etc. [45]. Thus, depending on the affinity of the drug or biomolecule for the nanocarriers surface, the zeta potential of the particles remains unaffected (low affinity) or drastically changes (high affinity), even leading to the reversion from positive to negative values [46]. The intercalation of MTX is produced without significant changes of the zeta potential [17,28] and the particle size distribution. On the contrary, DNA plasmids incorporation is produced on the surface, wrapping the LDH nanoparticles, reducing their zeta potential and increasing their particle size [37].

Table 2: Effect of the composition on the particle size and zeta potential of LDH nanoparticles.¡

Composition	**Hydrotermal treatment**	**Diameter (nm)**	**Zeta potencial (mV)**	**References**
MgAl-Cl-LDH	100° 16hours	116	40	[39]
CoAl-Cl-LDH	100° 16hours	127	38	[27]
$MgAl_{0.6}Fe^{III}_{0.4}$-Cl-LDH	100° 16hours	110	39	[27]
$MgAl-CO_3$-LDH	100° 16hours	71	45	[27]
$MgAl-NO_3$-LDH	100° 16hours	120	36	[37]
MgAl-Cl-LDH	100° 16hours	115	43	[46]
MgAl-Cl-LDH dsDNA	100° 16hours	150	-37	[46]
MgAl-Cl-LDH	100° 16hours	129	48	[47]
MgAl-Cl-LDH siRNA	100° 16hours	138	-38	[47]
$MgAl-CO_3$-LDH	100° 24hours	97	20	[48]
MgAl-MTX-LDH	100° 24hours	102	6	[48]
MgAl-FITC-LDH	100° 24hours	116	-5	[48]

2.4. Other synthesis methods of LDH nanoparticles

Besides the separate nucleation and aging methods, other routes have been proposed to obtain reproducible LDH nanoparticles with tailored size. One of them involves the use of a double hydrophilic block copolymer (DHBC) [49] constituted by a metal complexating section of polyacrilic acid and a stabilizing block of polyacrilamide. The presence of the DHBC in the solution of the metal ions leaded to the formation of micelles containing Al^{3+} ions. Upon alkaline solution addition, $Al(OH)_3$ nuclei were first formed, and afterwards, LDH nanoparticles. Highly stable dispersions of LDH nanoparticles were obtained, with sizes diminishing from 530 nm to 60 nm when the DHBC /(Mg^{2+}+Al^{3+}) increased from 0.27 to 1.0, which was assigned to a surface stabilization due to the presence of DHBC.

On the other hand, LDH-MTX nanohybrids were obtained by synthesis in reverse microemulsions, the water droplets acting as microvessels [50]. The microemulsion consisted in ciclohexane as oil phase, Triton X-100 as surfactant and n-butanol as cosurfactant. Two different solutions,

containing either the metal ions or the hydroxyl anions and the drug, were used as aqueous phase. The solid was formed by simply mixing the microemulsion and submitting it to hydrothermal treatment at 80 ºC for 24 hours. The droplets diameter of the microemulsion and, consequently, the size of the synthesized nanoparticles, was regulated by the water content.

3. Cellular release of drugs and genes by LDHs

An effective strategy for the cellular delivery of drugs using nanoparticles must consider, in the first place, their circulation from the point of insertion to the target cells [51]. Once the target is reached, there three barriers to achieve an optimal pharmaceutical action of the loading: transfection of the cellular membrane, release of the nanoparticles into the cytoplasm and detachment of the drug from the matrix. Moreover, in the case of genetic therapies, the loading should be able to transpose the nuclear membrane and accumulate in the nucleus [51]. In the case of LDH nanohybrids (Figure 3), the inorganic matrix not only provides the structure and anion binding sites to incorporate and protect the pharmaceutical agent, but also their physicochemical properties, which allow beating the above described barriers [17,33]. LDH nanoparticles portray a positively charged, hydrophillic surface to interact with the negatively charged cellular membrane [52]. The transfection of the cellular membrane is mediated by clathrin receptors, leading to endosome entrapment of the nanohybrid [47,48]. Once internalized, LDH nanohybrids may follow either an exocytic (endosomes-Golgi apparatus) or an endocytic (early endosome-late endosome-lysosome) pathway. In the latter, H^+ ions are pumped from the cytoplasm into the vesicle and eventually, the late endosome merges with the lysosome (pH 5.0–5.5), transferring its contents for degradation by lysosomal enzymes [53]. Both routes are common in the transport, storage, release and degradation of molecules bounded by the cells. However, the latter one is usually undesirable for drug delivery purposes, as the hydrolysis enzymes contained in the lysosomes may degrade the cargo.

In the case of LDH-drug nanohybrids, the influxing H^+ ions partly dissolve the nanoparticles in the late endosomes, which keeps the endosomal pH at 5–6, and hinder the lysosomal pathway [54]. More importantly, this neutralization reaction of LDH nanoparticles results in the release of Mg^{2+},Al^{3+} ions and the interlaminar anion. As the dissolution reaction proceeds, increasing number of ions are released within the endosome which causes water molecules, driven by osmotic forces, to enter from the cytoplasm into the endosome. This results in the osmotic swelling of the endosomal vesicles, which eventually burst and release residual LDH nanoparticles into the cytoplasm [33]. Finally, the drug release is producedby anion exchange or matrix dissolution. , but different authors [34,51] have proposed that the latter is predominant. This release mechanism presents, besides drug detachment and the buffering effect just mentioned, the advantage of avoiding the nanocarrier accumulation observed with other inorganic nanovehicles [51].

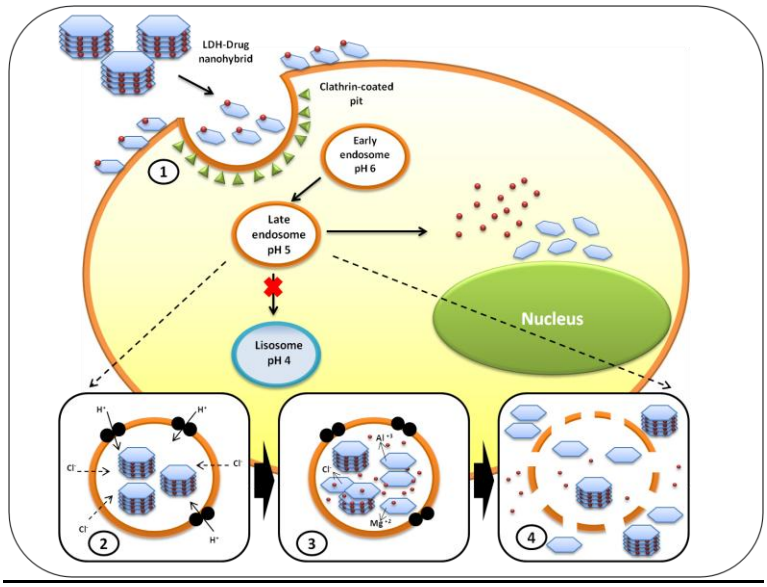

Figure 3: Schematic illustration of LDH-drug nanohybrids endocytosis and cellular trafficking. (1) LDH-drug nanohybrids attach to the cell membrane and clathrin-mediated endocytosis; (2) pumping H^+ ions into the endosome; (3) dissolution of LDH-drug nanohybrids; (4) entrance of water molecules and endosome burst.

Due to these advantages, LDH nanohybrids exhibit better performance than commercial transfection agents, such as Lipofectamine®. Ladewig and coworkers [17] found that siRNA transfection using LDH nanovehicles with an average diameter around 100 nm was more effective than this commercially available transfection agent. More than 99 % of the cells treated with an ERK2 protein silencing siRNA vehiculized by LDH nanoparticles were positive after 24 hours transfection and confocal microscopy confirmed the endocytotic uptake of LDH nanoparticles and the perinuclear localization of the siRNA in the cytoplasm. Moreover, the nanohybrid produced a substantial knockdown of ERK2 protein expression in human cells. Also, *in vivo* experiments showed the increased efficacy of LDH-MTX nanocarriers compared to the pure drug for the inhibition of colon cancer cells [55].

Finally, experiments in mice bearing orthotropic human breast tumors showed that LDH-MTX nanohybrids induced a 74.3% reduction of the tumor volume compared to pure MTX. This improved efficiency was explained by the higher targeting effect of the vehiculized MTX, the tumor to liver distribution ratio being 6-fold higher than that of the pure drug. As a result of the improved tumor-inhibitory effect, an increase of the survival rate was obtained: all tumor-bearing mice injected with the LDH-MTX nanohybrid survived 32 days after the treatment while only 66 % and 16.6 % survived when treated with pure MTX and untreated, respectively.

Nevertheless, the particle size control is essential to obtain an optimized performance of LDH nanoparticles. Their transfection capacity diminishes for nanoparticles larger than 200 nm [38,52], and particles of this size are also expected to be rapidly captured by the reticule endothelial system. On the other hand, excessively small particles lead to a fast release of the loading and,

consequently, a quick decrease of the drug concentration in the cellular medium [38]. Nanohybrids with average diameter lower than 100 nm also show higher cytotoxicity [34], causing a lower proliferation and membrane damage. This increased cytotoxicity has been attributed to the higher capacity of internalization and larger instability in the cellular media of small LDH nanoparticles. Then, it can be concluded that the optimal diameter of LDH nanocarriers ranges between 100 nm and 200 nm [52]. LDH nanohybrids in this size range present negligible cytotoxic effects up to a 250 μg/mL concentration in different normal and tumoral cell lines, while, at higher concentrations, celular membrane damage, oxidative stress and inflammatory response is produced [56]. These effects are more evident in tumoral than in normal cells and less acute than those measured with similar concentrations of other inorganic nanoparticles (iron oxides, silica and carbon nanotubes) [52,56].

Controlling the size and the morphology of the nanohybrids allows even the localization of the drug delivery to subcellular compartments, as demonstrated by Xu and coworkers [33]. These authors found that LDH-FITC nanohybrids with hexagonal (50-150 nm lateral size) morphology released the fluorescent probe in the perinuclear area whereas those with a rod (30-60 nm wide, 100-200 nm long) shape did it in the nucleus. The nuclear release produced by the rod shaped nanohybrids was assigned to their ability to pass through the cylinder-like pores of the nuclear membrane.

4. LDHs (bio)functionalization.

The ability to manipulate and control the surface properties of the nanocarriers is of paramount importance in order to produce new, effective nanotherapies [1,2,57,58]. LDH nanocarriers, as other inorganic nanoparticles, are retained by the reticuloendothelial system and mainly accumulated in liver, spleen, kidney and, to a lower extent, lungs [52,59,60]. In order to achieve site-specific release to, for example, bone tissue [2], the surface (bio)functionalization of the nanohybrids is mandatory. The incorporation of species that specifically recognizes receptors overexpressed in tumoral cells (such as folic acid, proteins or polysaccharides [40]) on the surface of the nanohybrids will also grant higher selectivity to the delivery of anticancer drugs.

There are two basic strategies to biofunctionalize nanohybrids with biomolecules, such as proteins: covalent or non-covalent interactions [61,62]. Non-covalent interactions do not require modifications while comprising many drawbacks, like weak attachment, random orientation, and conformational perturbation, reducing the biological activity and, hence, the molecular recognition capabilities of the adsorbed biomolecule. The non-covalent biofunctionalization is based on various adsorption interactions such as hydrophobic, electrostatic, hydrogen bonds, π-stacking, and van der Waals either on pristine or modified nanoparticles and takes place fast and spontaneously [63–65]. Hydrophobic interactions appear as the result of the dehydration of apolar parts of the protein and the sorbent surfaces; they often lead to changes in the conformation of the adsorbed proteins. Upon adsorption, at one side of the protein molecule the surrounding aqueous solution is replaced by the nanoparticle. As a consequence, the delicate balance between intramolecular and water-protein interactions, which determine the 3D native structure, may be disrupted [66,67]. The conformation parameters that influence the protein-nanoparticle

interactions include the distribution of charge and apolar residues and the stability and flexibility of the structure of a particular biomolecule. They may affect the adsorption behavior in such a way as to prevent it (especially hydrophilic electrostatically repelling surfaces) or to induce the opening of the hydrophobic core of the protein upon adsorption.

Covalent biofunctionalization requires the modification of the nanoparticle and/or the protein structure that usually causes significant changes on both the physical properties of the surface and the 3D structure of the protein [61,62]. On the other hand, this biofunctionalization induces a strong and stable interaction between the protein and the nanoparticle, which is of fundamental importance to develop specific, reproducible, and reusable biofunctional surfaces. Reactive surface sites are incorporated by modifying the bare nanoparticle or by precoating it with self assembled monolayers, polymers, dendrimers, etc., with different functional groups [68]. Reactive surface groups covalently bind proteins through one of the numerous functional groups of the side chains (ε-amino groups of lysine, β- and γ-carboxyl groups of aspartic and glutamic acids, and sulfhydryl groups of cysteine, phenolic, thiol, imidazole, and tyrosine residues). Since there may be multiple functional groups on the protein surface, adsorption via these reactive groups may cause different orientations and multipoint attachment causing a randomly oriented protein layer. The main advantage of this randomly oriented strategy is that the protein structure is not modified prior to the surface biofunctionalization. Consequently, this strategy is rather simple and mostly preserves the 3D conformation of native proteins. On the other hand, a variety of bioaffinity interactions based on different recognition principles (affinity ligand pairs and metal chelation) have been employed to induce site-oriented protein adsorption [69–72]. Among others, the bioaffinitiy is based on polyhistidine–metal cation, avidin–biotin, or protein A(G)–antibodies interactions.

Non-covalent biofunctionalization has been used to incorporate polymers, such as poly(ethylene glycol) (PEG), and proteins to LDH nanocarriers. PEG is the most widely used macromolecule to prolong the circulation half-life of nanocarriers and it also increases the stability towards aggregation and nonspecific binding to proteins and cells [1]. LDH-MTX nanoparticles were prepared by coprecipitation at variable pH process using PEG in the synthesis medium [28] and the separated aging step was performed in pure water. The PEG chains produced an additional stabilization of the LDH dispersions due to steric repulsions, while the particle size was slightly lower than for LDH nanoparticles synthesized in absence of PEG. Similar results were obtained when PEG/sebacate chains were adsorbed on the surface of LDH nanoparticles containing either nitrate or chloride [73]. The particle size did not significantly change after the PEG adsorption while their morphology was slightly modified, the PEG haired nanoparticles showing more rounded edges than that of pristine LDH. The PEG-haired nanoparticles exhibited a highly negative zeta potential and enhanced stability towards aggregation due to steric hindrance and electrostatic repulsion. Moreover, the solid obtained by freeze drying exhibited a higher redispersion capacity in water than the pristine LDH.

Proteins such as peptides, antibodies or enzymes are also attached to drug nanocarriers to provide stabilization and specificity [1]. In a recent work [74], we used bovine serum albumin (BSA) to

diminish the size of LDH particles and stabilize their dispersions. Positively charged LDH-Cl particles changed from micro to nano size when adsorbing BSA molecules at pH values higher than the isoelectric point of the protein (around pH 5). On the other hand, the low BSA adsorption on the negatively charged LDH-DS matrix was not enough to reduce the particle size. However, a fine tuning of the physicochemical properties of the LDH-Cl matrix by controlled DS^- incorporation, pH and ionic strength conditions leaded to LDH-BSA nanohybrids partially intercalated with the surfactant. BSA adsorption tailored the surface properties of both chloride and DS^- containing LDHs and also produced an increase of steric repulsions that hindered the nanocarriers agglomeration (Figure 4). LDH-BSA nanohybrids exhibited colloidal stability at high ionic strength (similar to that of biological fluids). These results indicated that, with a careful control of the biofunctionalization process, proteins can be incorporated to nanocarriers loaded with drugs featuring different physicochemical properties.

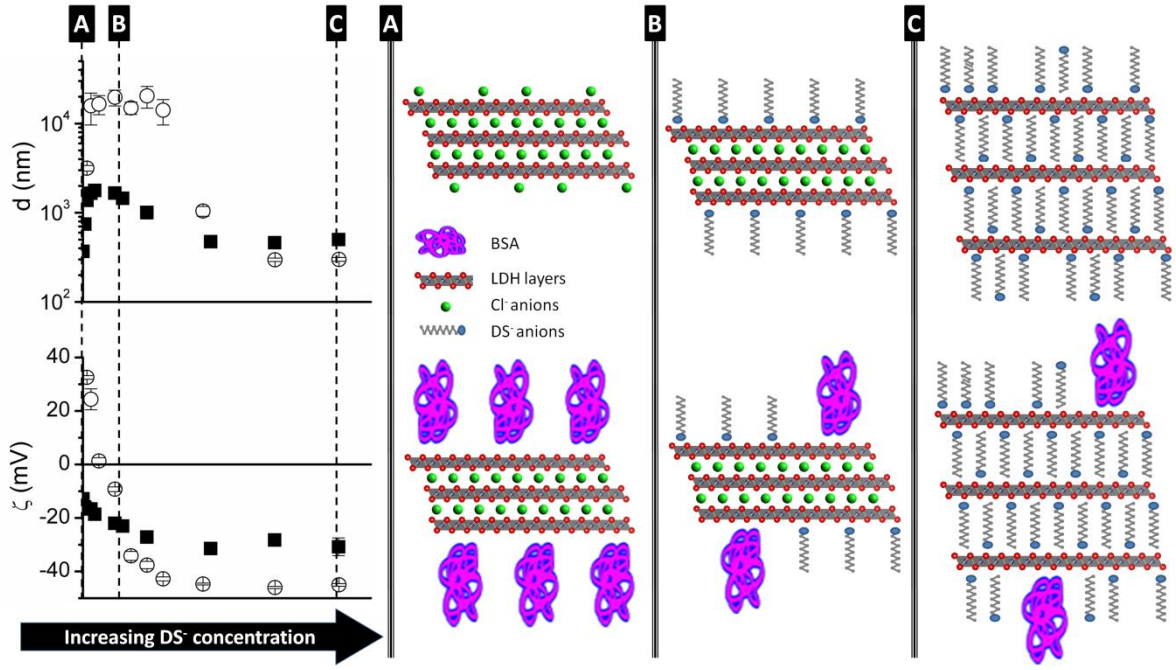

Figure 4: Left: zeta potential (ζ) and hydrodynamic diameter (d) variation with increasing dodecylsulfate (DS^-) concentration for LDH-Cl particles in the presence (squares) and in absence (circles) of BSA.Right: Eschematic representation of the LDH particles at different DS^- concentrations and in the presence (below) and absence (above of BSA).

The most common strategy to produce covalent biofuncionalization of LDH nanoparticles involves silanization of the surface, providing the reactive surface sites for the biomolecules. This process did not changed the size of LDH nanoparticles and it has been used to attach FITC for biodistribution and cellular internalization studies [38,54,75] or to tailor their surface properties

[57,59]. Further, this process has been used to impart targeting properties to LDH nanoparticles. Thus, silanization with aminopropyltrietoxysilane provided surface binding sites for the covalent coupling of folic acid (FA). This vitamin is a promising candidate for cancer-cell targeting as many human cancer cells overexpress FA receptors [76]. The transfection efficiency of the LDH-FA nanohybrids loaded with MTX was evaluated in KB (with over expression of FA receptors) and A549 (deficiency of FA receptors) cancer cell lines [77]. For KB cells, the intracellular MTX concentration obtained with the LDH-FA nanohybrid was 1.5 times higher than without the biofunctionalization, while the differences were negligible for A549 cells. It is important to note that proliferation and viability of KB and A549 cells were not affected in the presence of unloaded LDH-FA nanovehicles.

5. Bibliography

[1] J. Conde, J.T. Dias, V. Grazú, M. Moros, P. V Baptista, J.M. de la Fuente, Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine., Front. Chem. 2 (2014) 48.

[2] D. Wang, S.C. Miller, P. Kopecková, J. Kopecek, Bone-targeting macromolecular therapeutics., Adv. Drug Deliv. Rev. 57 (2005) 1049–76.

[3] J. Choy, J. Oh, S. Choi, Bio-inorganic Conjugates for Drug and Gene Delivery, (2008) 21–26.

[4] U. Costantino, V. Ambrogi, M. Nocchetti, L. Perioli, Hydrotalcite-like compounds: Versatile layered hosts of molecular anions with biological activity, Microp. Mesop. Mater. 107 (2008) 149–160.

[5] R. Rojas, M.C. Palena, A.F. Jimenez-Kairuz, R.H. Manzo, C.E. Giacomelli, Modeling drug release from a layered double hydroxide–ibuprofen complex, Appl. Clay Sci. 62-63 (2012) 15–20.

[6] V. Rives, M. del Arco, C. Martín, Intercalation of drugs in layered double hydroxides and their controlled release: A review, Appl. Clay Sci. 88-89 (2014) 239–269.

[7] F. Wypych, K.G. Satyanarayana, eds., Clay Surfaces: Fundamentals and applications, 2004.

[8] R. Rojas, Applications of Layered Double Hydroxides on environmental remediation, in: A.C. Carrillo, D.A. Griego (Eds.), Hydroxides Synth. Types Appl., Nova Science Publishers, New York, 2012: pp. 39–71.

[9] Q. Wang, D. O'Hare, Recent Advances in the Synthesis and Application of Layered Double Hydroxide (LDH) Nanosheets., Chem. Rev. 112 (2012) 4124–55.

[10] R. Rojas, M.R. Perez, E.M. Erro, P.I. Ortiz, M.A. Ulibarri, C.E. Giacomelli, EDTA modified LDHs as Cu2+ scavengers: removal kinetics and sorbent stability., J. Colloid Interf. Sci. 331 (2009) 425–31.

[11] M.A. Gonzalez, I. Pavlovic, R. Rojas-Delgado, C. Barriga, Removal of Cu2+, Pb2+ and Cd2+ by layered double hydroxide-humate hybrid. Sorbate and sorbent comparative studies, Chem. Eng. J. (2014).

[12] R. Rojas, Copper, lead and cadmium removal by Ca Al layered double hydroxides, Appl. Clay Sci. 87 (2014) 254–259.

[13] V. Rives, M. Del Arco, C. Martín, Layered double hydroxides as drug carriers and for controlled release of non-steroidal antiinflammatory drugs (NSAIDs): A review, J. Control. Release. 169 (2013) 28–39.

[14] J.-M. Oh, C.-B. Park, J.-H. Choy, Intracellular Drug Delivery of Layered Double Hydroxide Nanoparticles, J. Nanosci. Nanotechnol. 11 (2011) 1632–1635.

[15] H. Nakayama, K. Takeshita, M. Tsuhako, Preparation of 1-Hydroxyethylidene-1,1-diphosphonic Acid-Intercalated Layered Double Hydroxide and its Physicochemical Properties, 92 (2003) 2419–2426.

[16] M.S. Gasser, Inorganic layered double hydroxides as ascorbic acid (vitamin C) delivery system--intercalation and their controlled release properties., Colloids Surf. B. Biointerfaces. 73 (2009) 103–9.

[17] K. Ladewig, M. Niebert, Z.P. Xu, P.P. Gray, G.Q.M. Lu, Efficient siRNA delivery to mammalian cells using layered double hydroxide nanoparticles., Biomaterials. 31 (2010) 1821–9.

[18] Z. Gu, B.E. Rolfe, A.C. Thomas, J.H. Campbell, G.Q.M. Lu, Z.P. Xu, Cellular trafficking of low molecular weight heparin incorporated in layered double hydroxide nanoparticles in rat vascular smooth muscle cells., Biomaterials. 32 (2011) 7234–40.

[19] J. He, M. Wei, Y.-G. Kang, D.G. Evans, X. Duan, Preparation of layered double hydroxides, in: D.G. Evans, X. Duan (Eds.), Layer. Double Hydroxides, Springer-Verlag, Berlin, 2006: pp. 89–119.

[20] R. Rojas, F. Bruna, C.P. de Pauli, M.Á. Ulibarri, C.E. Giacomelli, The effect of interlayer anion on the reactivity of Mg-Al layered double hydroxides: improving and extending the customization capacity of anionic clays, J. Colloid Interf. Sci. 359 (2011) 136–141.

[21] H.-W. Olfs, L.O. Torres-Dorante, R. Eckelt, H. Kosslick, Comparison of different synthesis routes for Mg–Al layered double hydroxides (LDH): Characterization of the structural phases and anion exchange properties, Appl. Clay Sci. 43 (2009) 459–464.

[22] E.M. Seftel, E. Popovici, M. Mertens, K. De Witte, G. Van Tendeloo, P. Cool, et al., Zn–Al layered double hydroxides: Synthesis, characterization and photocatalytic application, Microporous Mesoporous Mater. 113 (2008) 296–304.

[23] J.S. Valente, M. Sánchez-Cantú, E. Lima, F. Figueras, Method for Large-Scale Production of Multimetallic Layered Double Hydroxides: Formation Mechanism Discernment, Chem. Mater. 21 (2009) 5809–5818.

[24] Y. Arai, M. Ogawa, Preparation of Co–Al layered double hydroxides by the hydrothermal urea method for controlled particle size, Appl. Clay Sci. 42 (2009) 601–604.

[25] T. Hibino, H. Ohya, Synthesis of crystalline layered double hydroxides: Precipitation by using urea hydrolysis and subsequent hydrothermal reactions in aqueous solutions, Appl. Clay Sci. 45 (2009) 123–132.

[26] Y. Zhao, F. Li, R. Zhang, D.G. Evans, X. Duan, Preparation of Layered Double-Hydroxide Nanomaterials with a Uniform Crystallite Size Using a New Method Involving Separate Nucleation and Aging Steps, Chem. Mater. 14 (2002) 4286–4291.

[27] Z.P. Xu, G. Stevenson, C.-Q. Lu, G.Q.M. Lu, Dispersion and size control of layered double hydroxide nanoparticles in aqueous solutions., J. Phys. Chem. B. 110 (2006) 16923–9.

[28] X.-Q. Zhang, M.-G. Zeng, S.-P. Li, X.-D. Li, Methotrexate intercalated layered double hydroxides with different particle sizes: structural study and controlled release properties., Colloids Surf. B. Biointerfaces. 117 (2014) 98–106.

[29] L. Li, W. Gu, J. Chen, W. Chen, Z.P. Xu, Co-delivery of siRNAs and anti-cancer drugs using layered double hydroxide nanoparticles., Biomaterials. 35 (2014) 3331–9.

[30] R. Rojas, A.F. Jimenez-Kairuz, R.H. Manzo, C.E. Giacomelli, Release kinetics from LDH-drug hybrids: Effect of layers stacking and drug solubility and polarity, Colloids Surfaces A Physicochem. Eng. Asp. 463 (2014) 37–43.

[31] M. Chen, H.M. Cooper, J.Z. Zhou, P.F. Bartlett, Z.P. Xu, Reduction in the size of layered double hydroxide nanoparticles enhances the efficiency of siRNA delivery., J. Colloid Interface Sci. 390 (2013) 275–81.

[32] Y. Wong, H.M. Cooper, K. Zhang, M. Chen, P. Bartlett, Z.P. Xu, Efficiency of layered double hydroxide nanoparticle-mediated delivery of siRNA is determined by nucleotide sequence., J. Colloid Interface Sci. 369 (2012) 453–9.

[33] Z.P. Xu, M. Niebert, K. Porazik, T.L. Walker, H.M. Cooper, A.P.J. Middelberg, et al., Subcellular compartment targeting of layered double hydroxide nanoparticles., J. Control. Release. 130 (2008) 86–94.

[34] S.-J. Choi, J.-M. Oh, J.-H. Choy, Safety Aspect of Inorganic Layered Nanoparticles: Size-Dependency In Vitro and In Vivo, J. Nanosci. Nanotechnol. 8 (2008) 5297–5301.

[35] H. Dong, M. Chen, S. Rahman, H.S. Parekh, H.M. Cooper, Z.P. Xu, Engineering small MgAl-layered double hydroxide nanoparticles for enhanced gene delivery, Appl. Clay Sci. 100 (2014) 66–75.

[36] H. Dong, H.S. Parekh, Z.P. Xu, Particle size- and number-dependent delivery to cells by layered double hydroxide nanoparticles., J. Colloid Interface Sci. 437 (2015) 10–6.

[37] K. Ladewig, M. Niebert, Z.P. Xu, P.P. Gray, G.Q. (Max) Lu, Controlled preparation of layered double hydroxide nanoparticles and their application as gene delivery vehicles, Appl. Clay Sci. 48 (2010) 280–289.

[38] J.-M. Oh, S.-J. Choi, G.-E. Lee, J.-E. Kim, J.-H. Choy, Inorganic metal hydroxide nanoparticles for targeted cellular uptake through clathrin-mediated endocytosis., Chem. Asian J. 4 (2009) 67–73.

[39] S. Yan, B.E. Rolfe, B. Zhang, Y.H. Mohammed, W. Gu, Z.P. Xu, Polarized immune responses modulated by layered double hydroxides nanoparticle conjugated with CpG., Biomaterials. 35 (2014) 9508–16.

[40] J.-M. Oh, S.-J. Choi, G.-E. Lee, S.-H. Han, J.-H. Choy, Inorganic Drug-Delivery Nanovehicle Conjugated with Cancer-Cell-Specific Ligand, Adv. Funct. Mater. 19 (2009) 1617–1624.

[41] R. Rojas Delgado, M. Arandigoyen Vidaurre, C.P. De Pauli, M.A. Ulibarri, M.J. Avena, Surface-charging behavior of Zn-Cr layered double hydroxide., J. Colloid Interf. Sci. 280 (2004) 431–41.

[42] R. Rojas, C. Barriga, C.P. De Pauli, M.J. Avena, Influence of carbonate intercalation in the surface-charging behavior of Zn–Cr layered double hydroxides, Mater. Chem. Phys. 119 (2010) 303–308.

[43] R. Rojas Delgado, C.P. De Pauli, C.B. Carrasco, M.J. Avena, Influence of MII/MIII ratio in surface-charging behavior of Zn–Al layered double hydroxides, Appl. Clay Sci. 40 (2008) 27–37.

[44] R. Rojas, C.E. Giacomelli, Effect of structure and bonding on the interfacial properties and the reactivity of layered double hydroxides and Zn hydroxide salts, Colloids Surf. A Physicochem. Eng. Asp. 419 (2013) 166–173.

[45] Z.P. Xu, Y. Jin, S. Liu, Z.P. Hao, G.Q.M. Lu, Surface charging of layered double hydroxides during dynamic interactions of anions at the interfaces., J. Colloid Interf. Sci. 326 (2008) 522–9.

[46] M. Chen, H.M. Cooper, J.Z. Zhou, P.F. Bartlett, Z.P. Xu, Reduction in the size of layered double hydroxide nanoparticles enhances the efficiency of siRNA delivery., J. Colloid Interface Sci. 390 (2013) 275–81.

[47] Y. Wong, K. Markham, Z.P. Xu, M. Chen, G.Q. Max Lu, P.F. Bartlett, et al., Efficient delivery of siRNA to cortical neurons using layered double hydroxide nanoparticles., Biomaterials. 31 (2010) 8770–9.

[48] J.-M. Oh, S.-J. Choi, S.-T. Kim, J.-H. Choy, Cellular uptake mechanism of an inorganic nanovehicle and its drug conjugates: Enhanced efficacy due to clathrin-mediated endocytosis., Bioconjug. Chem. 17 (2006) 1411–7.

[49] N. Sanson, F. Bouyer, M. Destarac, M. In, C. Gérardin, Hybrid polyion complex micelles formed from double hydrophilic block copolymers and multivalent metal ions: size control and nanostructure., Langmuir. 28 (2012) 3773–82.

[50] Z.-L. Liu, D.-Y. Tian, S.-P. Li, X.-D. Li, T.-H. Lu, MTX/LDHs hybrids synthesized from reverse microemulsions: particle control and bioassay study., Int. J. Pharm. 473 (2014) 414–25.

[51] K. Ladewig, Z.P. Xu, G.Q.M. Lu, Layered double hydroxide nanoparticles in gene and drug delivery., Expert Opin. Drug Deliv. 6 (2009) 907–22.

[52] S.-J. Choi, J.-H. Choy, Layered double hydroxide nanoparticles as target-specific delivery carriers: uptake mechanism and toxicity., Nanomedicine (Lond). 6 (2011) 803–14.

[53] L.K. Medina-Kauwe, J. Xie, S. Hamm-Alvarez, Intracellular trafficking of nonviral vectors., Gene Ther. 12 (2005) 1734–51.

[54] H.-E. Chung, D.-H. Park, J.-H. Choy, S.-J. Choi, Intracellular trafficking pathway of layered double hydroxide nanoparticles in human cells: Size-dependent cellular delivery, Appl. Clay Sci. 65-66 (2012) 24–30.

[55] J. Chakraborty, S. Roychowdhury, S. Sengupta, S. Ghosh, Mg-Al layered double hydroxide-methotrexate nanohybrid drug delivery system: evaluation of efficacy., Mater. Sci. Eng. C. Mater. Biol. Appl. 33 (2013) 2168–74.

[56] S.-J. Choi, J.-M. Oh, J.-H. Choy, Toxicological effects of inorganic nanoparticles on human lung cancer A549 cells., J. Inorg. Biochem. 103 (2009) 463–71.

[57] H. Hu, K.M. Xiu, S.L. Xu, W.T. Yang, F.J. Xu, Functionalized layered double hydroxide nanoparticles conjugated with disulfide-linked polycation brushes for advanced gene delivery., Bioconjug. Chem. 24 (2013) 968–78.

[58] X. Wang, Y. Yang, H. Jia, W. Jia, S. Miller, B. Bowman, et al., Peptide decoration of nanovehicles to achieve active targeting and pathology-responsive cellular uptake for bone metastasis chemotherapy, Biomater. Sci. 2 (2014) 961.

[59] P.-R. Wei, S.-H. Cheng, W.-N. Liao, K.-C. Kao, C.-F. Weng, C.-H. Lee, Synthesis of chitosan-coated near-infrared layered double hydroxide nanoparticles for in vivo optical imaging, J. Mater. Chem. 22 (2012) 5503.

[60] A.U. Kura, M.Z. Hussein, S. Fakurazi, P. Arulselvan, Layered double hydroxide nanocomposite for drug delivery systems; bio-distribution, toxicity and drug activity enhancement., Chem. Cent. J. 8 (2014) 47.

[61] C.E. Giacomelli, L.E. Valenti, Biomolecules and Solid Substrate Interaction : Key Factors in Developing Biofunctional Surfaces, in: P. Somasundaran (Ed.), Encycl. Surf. Colloid Sci., Taylor and Francis, 2011: pp. 1–16.

[62] M.F. Mora, L.E. Valenti, C.D. García, C.E. Giacomelli, Driving Forces and Consequences of the Adsorption of Proteins to Carbon Nanotubes, in: M. Vallet-Regí, M. Vila-Juarez (Eds.), Key Eng. Mater. Adv. Bioceram. Med. Appl., Trans Tech Publications, 2010: pp. 75–94.

[63] L.E. Valenti, P. a Fiorito, C.D. García, C.E. Giacomelli, The adsorption-desorption process of bovine serum albumin on carbon nanotubes., J. Colloid Interface Sci. 307 (2007) 349–56.

[64] M.F. Mora, C.E. Giacomelli, C.D. Garcia, Interaction of D-amino acid oxidase with carbon nanotubes: implications in the design of biosensors., Anal. Chem. 81 (2009) 1016–22.

[65] E. Herrera, C.E. Giacomelli, Surface coverage dictates the surface bio-activity of D-amino acid oxidase., Colloids Surf. B. Biointerfaces. 117 (2014) 296–302.

[66] C. Giacomelli, M. Bremer, W. Norde, ATR-FTIR Study of IgG Adsorbed on Different Silica Surfaces., J. Colloid Interface Sci. 220 (1999) 13–23.

[67] A.W.P. Vermeer, C.E. Giacomelli, W. Norde, Adsorption of IgG onto hydrophobic te £ on . Di ¡ erences between the F ab and F c domains, 1526 (2001) 61–69.

[68] L.S. Wong, F. Khan, J. Micklefield, Selective covalent protein immobilization: strategies and applications., Chem. Rev. 109 (2009) 4025–53.

[69] a Kausaite-Minkstimiene, a Ramanaviciene, J. Kirlyte, a Ramanavicius, Comparative study of random and oriented antibody immobilization techniques on the binding capacity of immunosensor., Anal. Chem. 82 (2010) 6401–8.

[70] L.E. Valenti, E. Herrera, F. Stragliotto, V.L. Martins, R.M. Torresi, C.E. Giacomelli, et al., Optimizing the Bioaffinity Interaction between His-Tag Proteins and Ni (II) Surface Sites, in: T. Horbett, J.L. Brash, W. Norde (Eds.), Proteins Interfaces III State Art, 2012: pp. 37–53.

[71] L.E. Valenti, V.L. Martins, E. Herrera, R.M. Torresi, C.E. Giacomelli, Ni(ii)-modified solid substrates as a platform to adsorb His-tag proteins, J. Mater. Chem. B. 1 (2013) 4921.

[72] L.E. Valenti, A.M. Smania, C.P. De Pauli, C.E. Giacomelli, Driving forces for the adsorption of a His-tag Chagas antigen. A rational approach to design bio-functional surfaces., Colloids Surf. B. Biointerfaces. 112 (2013) 294–301.

[73] D. Li, X. Xu, J. Xu, W. Hou, Poly(ethylene glycol) haired layered double hydroxides as biocompatible nanovehicles: Morphology and dispersity study, Colloids Surfaces A. 384 (2011) 585–591.

[74] C.E. Giacomelli, R. Rojas, Size-tunable LDH-protein hybrids aiming at the optimization of drug nanocarriers, Unpubl. Results. (n.d.).

[75] S.-J. Choi, J.-M. Oh, J.-H. Choy, Biocompatible ceramic nanocarrier for drug delivery with high efficiency, J. Ceram. Soc. Japan. 117 (2009) 543–549.

[76] S. Kularatne, P. Low, Targeting of Nanoparticles: Folate Receptor, in: S.R. Grobmyer, B.M. Moudgil (Eds.), Cancer Nanotechnol. SE - 17, Humana Press, 2010: pp. 249–265.

[77] D.J. Bharali, D.W. Lucey, H. Jayakumar, H.E. Pudavar, P.N. Prasad, Folate-Receptor-Mediated Delivery of InP Quantum Dots for Bioimaging Using Confocal and Two-Photon Microscopy, (2005) 11364–11371.